in the other reactions with KCl at or above 25 mM indicates that concentrations in the full range (*i.e.*, 25-200 mM) may be chosen if it is so desirable for any particular reaction conditions.

As shown in Figure 42, the invader-directed cleavage reaction requires the presence of salt (*e.g.*, KCl) for effective cleavage to occur. In other reactions, it has been found that KCl can inhibit the activity of certain Cleavase® enzymes when present at concentrations above about 25 mM (For example, in cleavage reactions using the S-60 oligonucleotide shown in Figure 30, in the absence of primer, the Cleavase® BN enzyme loses approximately 50% of its activity in 50 mM KCl). Therefore, the use of alternative salts in the invader-directed cleavage reaction was examined. In these experiments, the potassium ion was replaced with either Na^+ or Li^+ or the chloride ion was replaced with glutamic acid. The replacement of KCl with alternative salts is described below in sections c-e.

c) NaCl Titration

Figure 43 shows the results of using various concentrations of NaCl in place of KCl (lanes 3-10) in combination with the use 2 mM $MnCl_2$, in an otherwise standard reaction, in comparison to the effects seen with 100 mM KCl (lanes 1 and 2). The reactions analyzed in lanes 3 and 4 contained NaCl at 75 mM, lanes 5 and 6 contained 100 mM, lanes 7 and 8 contained 150 mM and lanes 9 and 10 contained 200 mM. These results show that NaCl can be used as a replacement for KCl in the invader-directed cleavage reaction (*i.e.*, the presence of NaCl, like KCl, enhances product accumulation).

d) LiCl Titration

Figure 44 shows the results of using various concentrations of LiCl in place of KCl (lanes 3-14) in otherwise standard reactions, compared to the effects seen with 100 mM KCl (lanes 1 and 2). The reactions analyzed in lanes 3 and 4 contained LiCl at 25 mM, lanes 5 and 6 contained 50 mM, lanes 7 and 8 contained 75 mM, lanes 9 and 10 contained 100 mM, lanes 11 and 12 contained 150 mM and lanes 13 and 14

contained 200 mM. These results demonstrate that LiCl can be used as a suitable replacement for KCl in the invader-directed cleavage reaction (*i.e.*, the presence of LiCl, like KCl, enhances product accumulation).

e) KGlu Titration

Figure 45 shows the results of using a glutamate salt of potassium (KGlu) in place of the more commonly used chloride salt (KCl) in reactions performed over a range of temperatures. KGlu has been shown to be a highly effective salt source for some enzymatic reactions, showing a broader range of concentrations which permit maximum enzymatic activity [Leirmo *et al.* (1987) Biochem. 26:2095]. The ability of KGlu to facilitate the annealing of the probe and invader oligonucleotides to the target nucleic acid was compared to that of LiCl. In these experiments, the reactions were run for 15 minutes, rather than the standard 20 minutes. The reaction analyzed in lane 1 contained 150 mM LiCl and was run at 65°C; the reactions analyzed in lanes 2-4 contained 200 mM, 300 mM and 400 mM KGlu, respectively and were run at 65°C. The reactions analyzed in lanes 5-8 repeated the array of salt concentrations used in lanes 1-4, but were performed at 67°C; lanes 9-12 show the same array run at 69°C and lanes 13-16 show the same array run at 71°C. The results shown in Figure 45 demonstrate that KGlu was very effective as a salt in the invasive cleavage reactions. In addition, these data show that the range of allowable KGlu concentrations was much greater than that of LiCl, with full activity apparent even at 400 mM KGlu.

f) $MnCl_2$ And $MgCl_2$ Titration And Ability To Replace $MnCl_2$ With $MgCl_2$

In some instances it may be desirable to perform the invasive cleavage reaction in the presence of Mg^{2+}, either in addition to, or in place of Mn^{2+} as the necessary divalent cation required for activity of the enzyme employed. For example, some common methods of preparing DNA from bacterial cultures or tissues use $MgCl_2$ in solutions which are used to facilitate the collection of DNA by precipitation. In addition, elevated concentrations (*i.e.*, greater than 5 mM) of divalent cation can be

used to facilitate hybridization of nucleic acids, in the same way that the monovalent salts were used above, thereby enhancing the invasive cleavage reaction. In this experiment, the tolerance of the invasive cleavage reaction was examined for 1) the substitution of $MgCl_2$ for $MnCl_2$ and for the ability to produce specific product in the presence of increasing concentrations of $MgCl_2$ and $MnCl_2$.

Figure 46 shows the results of either varying the concentration of $MnCl_2$ from 2 mM to 8 mM, replacing the $MnCl_2$ with $MgCl_2$ at 2 to 4 mM, or of using these components in combination in an otherwise standard reaction. The reactions analyzed in lanes 1 and 2 contained 2 mM each $MnCl_2$ and $MgCl_2$, lanes 3 and 4 contained 2 mM $MnCl_2$ only, lanes 5 and 6 contained 3 mM $MnCl_2$, lanes 7 and 8 contained 4 mM $MnCl_2$, lanes 9 and 10 contained 8 mM $MnCl_2$. The reactions analyzed in lanes 11 and 12 contained 2 mM $MgCl_2$ and lanes 13 and 14 contained 4 mM $MgCl_2$. These results show that both $MnCl_2$ and $MgCl_2$ can be used as the necessary divalent cation to enable the cleavage activity of the Cleavase® A/G enzyme in these reactions and that the invasive cleavage reaction can tolerate a broad range of concentrations of these components.

In addition to examining the effects of the salt environment on the rate of product accumulation in the invasive cleavage reaction, the use of reaction constituents shown to be effective in enhancing nucleic acid hybridization in either standard hybridization assays (*e.g.*, blot hybridization) or in ligation reactions was examined. These components may act as volume excluders, increasing the effective concentration of the nucleic acids of interest and thereby enhancing hybridization, or they may act as charge-shielding agents to minimize repulsion between the highly charged backbones of the nucleic acids strands. The results of these experiments are described in sections g and h below.

g) Effect Of CTAB Addition

The polycationic detergent cetyltrietheylammonium bromide (CTAB) has been shown to dramatically enhance hybridization of nucleic acids [Pontius and Berg (1991) Proc. Natl. Acad. Sci. USA 88:8237]. The data shown in Figure 47 depicts the results